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Investigation of DKK-1 (DICKKOPF-1) and DKK-3 (DICKKOPF-3) levels before and after cardiopulmonary bypass

DKK-1 and DKK-3 levels in coronary artery disease

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Abstract
Aim: According to recent studies related to coronary artery diseases, Dkk-1(Dickkopf-1) and Dkk-3(Dickkopf-3) levels in the blood have been found to be associated with the onset and progression of atherosclerosis. This study was carried out to investigate Dkk-1 and Dkk-3 levels in patients who underwent surgery with the cardiopulmonary bypass method.
Material and Methods: Thirty patients who were operated on by the cardiopulmonary bypass method were included in this study. Venous blood samples were taken from the patients before and after cardiopulmonary bypass, and Dkk-1 and Dkk-3 proteins were measured using the ELISA method.
Results: As a result of the study, a statistically significant difference was observed between Dkk-1 values measured before and after cardiopulmonary bypass ($p<0.01$). Likewise, there was a statistically significant difference between Dkk-3 values measured before and after cardiopulmonary bypass ($p<0.05$).
Discussion: The decrease in Dkk-1 and Dkk-3 levels before cardiopulmonary bypass after surgery shows that these values are important in terms of protection against atherosclerosis. With these parameters, which have been observed to have protective effects against the formation of atherosclerosis and against atherosclerosis, possible heart damage can be prevented with therapeutic strategies that reduce myocardial damage.

Keywords
Atherosclerosis, Dkk-1(Dickkopf-1), Dkk-3(Dickkopf-3), Cardiopulmonary Bypass

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Introduction

Coronary artery disease (CAD) is the third leading cause of death worldwide and is associated with 17.8 million deaths per year [1].

Atherosclerosis is a progressive inflammatory disease of the arterial wall that can remain clinically asymptomatic for years before triggering acute attacks such as myocardial infarction or stroke, which are among the first symptoms of CAD [2].

The condition has been reported to be generally associated with cholesterol deposition, macrophage infiltration, smooth muscle cell proliferation, connective tissue deposition, and the presence of a thrombus. In the early stages of atherosclerosis formation, thin fatty streaks form on the arterial wall due to endothelial cell damage. The accumulation of materials such as lipids and the proliferation of smooth muscle cells leads to the formation of advanced lesions known as atherosclerotic plaques containing fibrous layers of varying thickness. In humans, atherosclerotic plaques consisting of thin fibrous structures are irregular and in some cases break up, causing acute coronary syndromes [3].

The number of patients with ischemic heart disease (IHD) associated with CAD is also increasing, and IHD continues to be a significant health burden worldwide [4]. IHD manifests clinically as myocardial infarction and ischemic cardiomyopathy. IHD is the number one cause of death, disability, and human suffering globally. Age-adjusted rates show a promising decrease. However, health systems have to manage an increasing number of cases due to population aging [5].

There is a great need for studies aimed at finding effective ways for the prevention and treatment of CAD and to discover targeted molecules. In recent years, in studies on CAD, interest in primary prevention research has increased; studies on this subject are of great importance in reducing the incidence of CAD. Although many important advances have been made in the treatment of CAD, it is necessary to take steps to address the causes of the success of targeted therapies because it is important to determine the causes before applying a beneficial treatment [6]. Recently, new biomarkers have been investigated in addition to cardiac damage biomarkers, including Dkk-1 and Dkk-3.

Dkk-1, a founding member of the Dickkopf family, is a secreted glycoprotein most extensively characterized as an inhibitor of the canonical β-catenin-dependent Wnt pathway [7]. Wnt proteins regulate a variety of physiological processes including cell proliferation, differentiation, migration and apoptosis [8].

It acts by blocking 5 or 6 binding of Wnt ligands coreceptors to LRP (low-density lipoprotein receptor-related protein). This mode of action is not fully understood. However, this mechanism of action is indirect and involves the Wnt signal balance of Dkk-1 shifting from β-catenin-dependent pathways to β-catenin-independent pathways. Finally, in addition to binding to LRP5/6, Dkk-1 is a high-affinity ligand for the Krm (Kremen) 1 and 2 transmembrane proteins. The interaction between LRP6, Dkk-1 and Krm triggers LRP receptor internalization and degradation, thus providing an additional mechanism for Wnt signaling inhibition [9].

Dickkopf-3 (Dkk-3), on the other hand, appears to be a different member of the Dickkopf family. In contrast to the more closely related Dkk-1, 2 and 4, Dkk-3 is reported to be associated with Dickkopf-like protein 1, a potentially distant Dkk family member [10].

Human DKK-1, 2 and 4 are located on the same paralog chromosome set. However, DKK-3 is not part of this group. Also, unlike other members of the Dkk protein family, Dkk-3 does not affect the canonical Wnt/β-catenin pathway and does not bind to Krms, but rather regulates TGF-β signaling [11]. Moreover, in a pathological study in mice, DKK-3 was identified as a hormone that can induce endothelial cell migration, promote re-endothelialization, and prevent lesion formation in arterial vessels [12].

Like Dkk-1, Dkk-3 has been mainly investigated in oncology. It shows a suppressive effect in various types of cancer in humans and has been suggested to act therapeutically [10].

Material and Methods

Ethics committee approval

This study was approved by the Harran University Faculty of Medicine Clinical Research Ethics Committee, with the decision dated 30.12.2019, session numbered 08 and numbered 21.

Patients included in the study

This study was conducted in accordance with the Helsinki Declaration, which was revised in 1989. Thirty patients who were operated on by the cardiopulmonary bypass method were included in this study. Venous blood samples were obtained from the patients included in the study.

Obtaining Blood Plasma

In patients who underwent cardiac surgery with the CPB method, blood was drawn before and after CPB and put into sterile tubes with anticoagulant (heparin). The tube from which blood was drawn was immediately transferred to an ice-filled container and transported to the laboratory. The sterile tube was then centrifuged at 5000 rpm for 5 minutes. After the centrifugation step, the plasma, which is the supernatant part, was taken into an RNase-free tube (Eppendorf tube) and stored at -80 to be studied.

Analysis of Dkk-1(Dickkopf-1) and Dkk-3(dickkopf-3)

Enzyme-linked immunosorbent assay (ELISA) kit protocol was applied. The samples to be studied (Serum) were brought to room temperature at least 2 hours before. Samples in the 96-well plate were washed before adding, and standard/sample (100 µl) was added and incubated at 37°C for 90 minutes. The samples were removed and biotinylated detection antibody (100 µl) was added and incubated for 60 minutes. After washing, 100 µl of SABC working solution (HRP) was added to all wells. After 30 minutes of incubation, 90 µl of TMB substrate was added and when a visible color change was observed, stop solution (50 µl) was added and yellow color formation was observed. Optical density (OD) value at 450 nm (OD450 nm) was determined and the data were evaluated.

Measurement of Antioxidant and OS Parameters in Plasma

Total Antioxidant Status (TAS) Measurement

Measurement of (TAS in PS and plasma was performed using Rel Assay Diagnostics total oxidant capacity measurement kit (Rel Assay Diagnostics, Lot. No: HN20106A, Turkey). Plasma TAS levels were determined using a new automated measurement

method developed by Erel [13].

Total Oxidant Status (TOS) Measurement

Rel Assay Diagnostics total oxidant capacity measurement kit (Rel Assay Diagnostics, Lot. No: OK20115O, Turkey) was used for the measurement of TOS in PS and plasma. Plasma TOS levels were determined using a new automated measurement method developed by Erel [14].

Oxidative Stress Index (OSI) Measurement

OSI was calculated by dividing it as Total Oxidant Level (TOS)/ Total Antioxidant and expressed as Arbitrary Unit (AU).

Statistical analysis

The conformity of the data to the normal distribution was tested with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent Samples t-test was used for those with the normal distribution of numerical variables, the Mann-Whitney U test was used for comparisons of two independent groups for those who did not, and One-way analysis of variance (ANOVA) and LSD multiple comparison tests were used for normally distributed features in comparisons of non-normally distributed features in more than two independent groups. For features, the Kruskal-Wallis test and all pairwise multiple comparison tests were used. As descriptive statistics, mean±standard deviation for numerical variables, number and percentage values for categorical variables are given. SPSS Windows version 24.0 package program was used for statistical analysis and $p<0.05$ was considered statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

Demographic Data of the Working Group

Of the patients included in the study, 14 were female and 16 were male, with a mean age of 58 (years), height of 165.25 (cm), weight of 72.55 (kg) and body surface area (BSA) of 1.793 (m2) aspect calculated. The demographic characteristics of 30 patients included in the study are shown in Table 1.

Table 1. Demographic characteristics of the patients included in the study.

Variable	Patients (n=30)
Gender (Male/Female) %	% 14/16 (% 46,66 / % 53,34)
Age (years)	58 ± 8,67
Height (cm)	165,25 ± 7,35
Weight (kg)	72,55 ± 18,7 0
BSA (body surface area) (m^2)	1,784 ± 0,14

Table 2. Statistical Analysis of Pre- and Post-CPB Groups

	Before CPB			After CPB			P
	Min	Max	Mean±SS	Min	Max	Mean±SS	
TAS	0,81	1,97	1,5 ± 0,25	1,21	1,78	1,51 ± 0,15	0,829
TOS	14,23	28,72	19,67 ± 3,4	11,9	24,02	17,58 ± 2,99	0,014*
OSI	0,82	2,5	1,35 ± 0,36	0,85	1,74	1,17 ± 0,23	0,025*
DKK1	44,37	112,75	76,04 ± 14,34	31,64	83,16	56,13 ± 13,99	0,001**
DKK3	8,55	45,3	21,47 ± 10,33	10,2	43,24	17,9 ± 8,44	0,149

p<0,05, **p<0,001

Table 3. Correlation Analysis of Variables

		TAS	TOS	OSI	DKK-1	DKK-3
TAS	P	1	-0,01	-,637**	0	0,12
	r		0,95	0	0,97	0,38
TOS	p	-0,01	1	,742**	0,07	0,11
	r	0,95		0	0,59	0,4
OSI	p	-,637**	,742**	1	0,07	-0,01
	r	0	0		0,61	0,92
DKK-1	p	0	0,07	0,07	1	0,02
	r	0,97	0,59	0,61		0,87
DKK-3	p	0,12	0,11	-0,01	0,02	1
	r	0,38	0,4	0,92	0,87	

ELISA Results in Plasma

The results of the optical density reading at 450 nm using commercial kits for the level determination of Dkk-1 and Dkk-3 in plasma by ELISA method and the results of OS Parameters are shown in Table 2.

Biochemical analysis results of blood samples taken from patients before and after CPB at the values indicated in Table 2, TOS, OSI and DKK-1 values were found to be statistically significant (*$p<0.05$, $p<0.001$). When the total antioxidant capacity (TAS) of the patients before and after CPB was compared, a significant increase was observed.

According to the correlation result in Table 3, there was a moderately significant negative correlation between TAS value and OSI value. There is a highly significant positive correlation between the TOS value and the OSI value.

Discussion

Considering the conditions that cause ischemic heart disease, factors such as unstable angina, myocardial infarction and acute coronary syndrome causing sudden death, fragmentation of irregular thrombotic plaques and coronary atherosclerosis can be mentioned [15].

It has been found over the years that the composition and fragility of the plaque, rather than the size of the plaque or the severity of the stenosis, are important determinants in the development of acute coronary syndrome. Because soft irregular plaques are more prone to disintegration than collagen-rich hard plaques, they become thrombogenic after degradation [16].

One of the most important factors that should be evaluated for the development of atherosclerosis that may develop in the coronary vessels, as well as the development of CAD, is the follow-up of irreversible cell and tissue damage. As a result of not following this situation and not taking the necessary precautions, the processes starting from the cell can manifest itself deeply with organ damage and sudden death. Before reaching this stage, the cell or tissue synthesizes biomarkers that provide information about its state. For example, with the formation of atherosclerosis, proteins such as DKK-1 and DKK-3 can actively provide some information about the current situation. Therefore, it may be necessary to monitor these parameters at certain intervals before the development of atherosclerosis and to take measures accordingly. In our

study, pre- and postoperative Dkk-1(Dickkopf-1) and Dkk-3 (Dickkopf-3) levels of the patients were examined and the effect of the surgery on Dkk-1(Dickkopf-1) and Dkk-3 (Dickkopf-3) levels was investigated.

It is not known exactly how Dkk-1 protein affects the cardiovascular system. In a study by Ueland et al., they described the occurrence of increased Dkk-1 concentrations in patients with atherosclerotic disorders and in patients with symptomatic aortic stenosis [17].

Another study evaluated Dkk-1 levels in serum samples obtained at different times in a subgroup of approximately 5000 patients with acute coronary syndrome, based on the hypothesis that Dkk-1 may be associated with cardiovascular diseases. In the PLATO study, they reported that dual antiplatelet therapy (Platelet Inhibition and Patient Outcomes) was associated with a combination of cardiovascular death, myocardial infarction, or stroke, independent of the normal level of Dkk-1, in these patients [18]. In our study, the mean Dkk-1 value of the patients before CPB was 76.04 ng/ml, and the mean Dkk-1 value after CPB was 56.13 ng/ml. As a result of the study, it was observed that there was a statistically significant difference between the Dkk-1 values measured before and after CPB. This situation shows that the Dkk-1 value is important for the formation of atherosclerosis in parallel with other studies and that Dkk-1 level decreases after CPB.

Studies indicate that Dkk-1 is directly involved in proatherogenic states, suggesting that Dkk-1 activity may affect the pathophysiology of the arterial wall by modulating Wnt signaling. Guo et al. reported in their mouse studies that suppression of Wnt/β-catenin signaling by platelet-derived Dkk-1 causes acute lung injury and strengthens the infiltration of neutrophils into the pulmonary parenchyma [19].

A study using tandem stenosis model 13 vessel graft and rabbit models of atherosclerotic plaques was conducted to determine the therapeutic potential of DKK-3 in altering plaque composition. As a result of this study, they stated that DKK-3 has vascular effects associated with cardiovascular pathologies in addition to its strong tumor-suppressive effect [20].

The role of Dkk-3 in atherosclerosis has recently been discovered. In a prospective study, in contrast to Dkk-1, plasma Dkk-3 levels were inversely and independently associated with common carotid artery intima-media thickness and 5-year progression of carotid atherosclerosis, suggesting that this may protect against both early and advanced stages of atherogenesis stated [21].

In our study, the mean Dkk-3 (Dickkopf-3) value of the patients before CPB was 21.47 ng/ml, and the mean Dkk-3 (Dickkopf-3) value after CPB was 17.90 ng/ml. As a result of the study, it was observed that there was a statistically significant difference between Dkk-3 (Dickkopf-3) values measured before and after CPB. This shows that Dkk-3(Dickkopf-3) value is important in terms of protection against atherosclerosis, and Dkk-3(Dickkopf-3) level decreases after cardiopulmonary bypass.

When examined in terms of oxidative stress in our study, the mean preoperative OSI value was 1.35 ng/ml, the mean postoperative OSI value was 1.17 ng/ml. As a result of the study, it was observed that there was a statistically significant difference between the OSI values measured before and after the surgery. This shows us that the low mean postoperative OSI value is an important criterion for myocardial tissue damage and myocardial ischemia, and the OSI level decreases after cardiopulmonary bypass.

Conclusion

In the study, the protective effects of Dkk-1(Dickkopf-1) and Dkk-3(Dickkopf-3) against the formation of atherosclerosis and atherosclerosis were observed, and results were obtained in parallel with previous studies. Significant differences were observed in the levels of Dkk-1(Dickkopf-1) and Dkk-3(Dickkopf-3) proteins of the patients whose blood samples were taken before and after CPB. We can say that Dkk-1(Dickkopf-1) and Dkk-3(Dickkopf-3) proteins are important in cardiovascular diseases. In our study, mean Dkk-1(Dickkopf-1) values of the patients after CPB were 56.13 ng/ml, and these values were below the preoperative values. In this case, it was understood that the low Dkk-1(Dickkopf-1) value has a reducing effect on the formation of atherosclerosis. In our study, mean Dkk-3(Dickkopf-3) values of the patients after CPB were 17.90 ng/ml, and these values were below the preoperative values. This shows us that high Dkk-3(Dickkopf-3) values are important in preventing atherosclerosis.

It is anticipated that the results of this research, which examines the formation of atherosclerosis and the reducing effects of Dkk-1(Dickkopf-1) and Dkk-3(Dickkopf-3) proteins, will be a guide for scientists. In order for the research to be more inclusive, it is necessary to conduct research in larger sample groups.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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